A COMPARATIVE EVALUATION OF THE PERFORMANCE OF TWO NATURAL COAGULANTS (SEEDS OF *Strychnos potatorum* AND *Moringa oleifera*) IN DOMESTIC WATER PURIFICATION

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CERTIFICATE

Certified that the work presented in this thesis entitled "A Comparative Evaluation of the performance of Two Natural Coagulants (Seeds of *Strychnos potatorum* and *Moringa oleifera*) in Domestic Water Purification" by Shri C. Raveendra Babu has been carried out under my supervision and has not been submitted elsewhere for a degree.

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ABSTRACT

Performance of two natural coagulants (seeds of *Strychnos potatorum* and *Moringa oleifera*) was evaluated in terms of turbidity, bacteria and virus removal using the Lower Ganga canal water in the turbidity ranges 18-21, 38-42, and 130-135 NTU. For turbidity removal in batch coagulation-sedimentation test, optimum dosages of *S. potatorum* and *M. oleifera* seeds were 1.5 and 200 mg/L, respectively. Supernatant characteristics were poor (turbidity 15-25 NTU, and heterotrophic plate count bacteria removal 19.50-43.75%) for *S. potatorum*, whereas *M. oleifera* showed moderately good supernatant characteristics (turbidity 6.5-10.0 NTU, and heterotrophic plate count bacteria removal 84.38-94.41%). Both coagulants, however, showed excellent removal of seeded poliovirus (*S. potatorum* 99.92-99.97%; *M. oleifera* 99.93-99.96%). Coagulation-filtration test, consisting of mixing the optimum coagulant dose (based on supernatant turbidity in coagulation-sedimentation test) with low-turbidity sewage-spiked raw water (turbidity 15-25 NTU, plate count bacteria 245-493 CFU/mL, and fecal coliforms 220-500 MPN/100 mL) by hand-stirring (2-3 minutes) and filtration of the dosed water through sand (50 cm; 500-710 μm), yielded superior effluent characteristics with both seeds (*S. potatorum* : turbidity 0.37-1.60 NTU, plate count bacteria 4-13 CFU/mL, and fecal coliforms 3-11 MPN/mL; *M. oleifera* : turbidity 0.3-1.1 NTU, plate count bacteria 5-19 CFU/mL, and fecal coliforms 4-20 MPN/100 mL). Removal of seeded poliovirus was greater than two log for *S. potatorum* and three to four log for *M. oleifera*. However, *S. potatorum* seed produced higher throughput before filter backwashing became necessary. Infrared spectra and zeta potential studies indicated the coagulating action of *S. potatorum* seed as an anionic polyelectrolyte and *M. oleifera* seed as a cationic polyelectrolyte. The findings of the present study has re-established the usefulness of *S. potatorum* and *M. oleifera* seeds as natural coagulants. The simple coagulation-filtration method appeared feasible for domestic water purification in rural areas of developing countries.

1. INTRODUCTION

Safe and adequate water supply is of paramount importance to human health and well-being of the society as a whole. Water of poor quality can be the cause of a number of enteric diseases, e.g., cholera, typhoid, infectious hepatitis (jaundice), dysentery and gastroenteritis. These diseases cost millions of lives every year, especially in developing countries.

Nearly 75% of the present global population lives in the developing regions of the world; because the rate of increase of population in many such regions is also higher than that in the industrialised countries, the share of the developing world population will have increased to almost 80% by the year 2000 (WHO, 1981). The industrialised countries have eradicated the water-related diseases to a great extent through technical advancement, whereas safe and adequate drinking water supply is yet a luxury for majority of people in developing countries. The International Drinking Water Supply and Sanitation Decade (1981-90) was aimed at providing safe water to the entire population of the world. A major portion of the task in terms of quantity of water supply has been achieved by tapping the groundwater through handpumps. However, a vast majority of the rural population in developing countries is still dependent on surface water sources and the need for quality improvement of such supplies to protect public health has been well recognised. This has generated a renewed interest in the assessment of some of the

traditional domestic water purification methods such as the use of natural coagulants which is still in vogue in some rural areas of Asia and Africa. To disseminate available information (laboratory research and field application) about natural coagulants, an international seminar entitled "The Use of Natural Coagulants in Water Treatment" was organised by the Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH, Germany in Yogyakarta, Indonesia during 2-7 October, 1989. The seminar recommended studies on comparative evaluation of the performance of natural coagulants in water purification under similar conditions.

In India, the seed of *Strychnos potatorum* (Nirmali seed) is still in use in rural areas of Tamil Nadu and Maharashtra in domestic water purification. The seed of *Moringa oleifera*, popular as a natural coagulant in African villages, has never been used in water purification in India even though it is abundant all over the country. Recently, the Voluntary Health Association of India (VHAI) has initiated efforts to popularise the use of the seeds in domestic water purification in rural areas.

The present laboratory study was directed towards a comparative evaluation of the performance of the seeds of *S. potatorum* and *M. oleifera* in domestic water purification in terms of turbidity, bacteria and virus removal.

2. LITERATURE REVIEW

This chapter reviews the pertinent literature——drinking water quality for developing countries and domestic water purification by natural coagulants——to provide a background for the study.

2.1 Drinking Water Quality for Developing Countries

The most common and widespread danger associated with drinking water is contamination, either directly or indirectly by sewage, human and animal excrement or other wastes. If such contamination is recent, and if among the contributors there are carriers of communicable enteric diseases, some of the living causal agents may be present. The drinking of water so contaminated or its use in the preparation of certain foods may result in further cases of infection (WHO, 1984a).

In developed countries, with virtual disappearance of waterborne diseases, more attention is being directed towards the public health effects of chronic diseases resulting from the presence of low concentrations of organic chemicals in drinking water, e.g., chlorinated hydrocarbons. In developing countries where the enteric diseases are predominant health hazards arising from drinking water quality, emphasis must be placed on the microbiological quality because health risk due to chemical substances present in water is usually at a much lower level compared to health hazards due to microbiological contaminants (Bradley, 1977).

Turbidity in water is caused by the presence of suspended matter, such as clay, silt, colloidal organic particles, and microscopic organisms. The particles that cause turbidity in water range in size from colloidal dimensions (ca. 10 nm) to diameters of the order of 0.1 mm. Although the clay or other inert suspended particles which are the main causes for turbidity may not be harmful to health, yet they are to be removed or reduced for aesthetic and psychological reasons. Further, the presence of turbidity can have a significant effect on the microbiological quality of drinking water. The detection of bacteria and viruses in drinking water may be complicated by the presence of turbidity. In water, microbial growth is most extensive on the surfaces of particles and inside loose, naturally occurring floc. This growth is facilitated because nutrients are adsorbed onto surfaces and attached bacteria are thus able to grow more efficiently compared to discrete organisms. The adsorptive capacity of some suspended particulates can lead to the entrapment of undesirable inorganic and organic compounds present in the water and in this way, turbidity can bear an indirect relationship to the health aspects of water quality (WHO, 1984a). The recommended guideline value for turbidity in drinking water is 5 nephelometric turbidity units (NTU), but levels should preferably be less than 1 NTU when disinfection is practiced (WHO, 1984).

Among the various microorganisms present in water, those that are of public health significance are the intestinal

pathogens. Intestinal bacterial pathogens are widely distributed throughout the world. Those known to have occurred in contaminated drinking water include strains of *Salmonella typhi*, *Shigella flexneri*, enterotoxigenic *Escherichia coli*, *Vibrio cholerae*, *Yersinia enterocolitica* and *Campylobacter fetus*. These organisms may cause diseases that vary in severity from mild gastroenteritis to severe and sometimes fatal dysentery, cholera, or typhoid (WHO, 1984a). Viruses of major concern are essentially those that multiply in the intestine and are excreted in the feces of the infected individuals—the enteric viruses, such as hepatitis A virus, poliovirus, and rotavirus.

Among the various waterborne pathogens, there exists a wide range of minimum infectious dose levels necessary to cause a human infection. The size of the infective dose also varies in different persons with age, nutritional status, and general health at the time of exposure. Although it is possible to detect the presence of many pathogens in water, the methods of isolation and enumeration are often complex and time consuming. Therefore, it is impracticable to monitor drinking water for every possible microbial pathogen that might occur with contamination. A more logical approach is the detection of organisms normally present in the feces of man and other warm blooded animals as indicators of excremental pollution, as well as of the efficacy of water treatment. The primary bacterial indicator recommended for this purpose is the coliform group of organisms as a whole; detection of fecal (thermotolerant) coliforms, in particular *E. coli*,

provides definite evidence of fecal pollution. Table 1 shows the guideline values for bacteriological quality of drinking water recommended by WHO (WHO, 1984). To be acceptable, drinking water should be free from the enteric viruses; however, virological safety of a water cannot be assessed in an absolute sense because neither the available monitoring techniques nor epidemiological evaluation is sufficiently sensitive to ensure the absence of viruses (WHO, 1984a). Under these circumstances, researchers prefer to use model enteric viruses to evaluate the efficacy of water treatment methods in reducing virus transmission.

Bacterial growth is a major problem both in water distribution systems (Geldreich *et al*, 1972) and in point-of-use water treatment devices (Geldreich *et al*, 1985). Many regulatory agencies have proposed that the total bacterial count in finished water should not exceed 500 colony-forming units (CFU)/mL in order to reduce interference with the detection of coliforms and to reduce subsequent potential health risk. A recent finding by Payment *et al* (1991), correlating heterotrophic plate count (membrane filtration method - 35 $\pm$ 0.5^{o}C/48 $\pm$ 3 hours - R2A agar) of point-of-use devices and reported gastrointestinal symptoms, has further increased significance of monitoring such bacterial population in drinking water.

2.2 Domestic Water Purification by Natural Coagulants

Many natural polyelectrolytes of plant origin can be used to clarify muddy water. For domestic use, the materials have to be used in the form of crude plant powder suspensions, 90% of which

Table 1. Guideline Values for Bacteriological Quality of Drinking Water (WHO, 1984)

Organism	Guideline Value (Number/100 mL)	Remarks
Piped Water Supplies		
Treated Water Entering the Distribution System		
Fecal Coliforms	0	turbidity <1 NTU; for disinfection with chlorine, pH preferably <8.0, free chlorine residual 0.2–0.5 mg/L following 30 minutes (minimum) contact
Coliforms	0	
Untreated Water Entering the Distribution System		
Fecal Coliforms	0	
Coliforms	0	in 98% of samples examined throughout the year – in the case of large supplies when sufficient samples are examined
Coliforms	3	in an occasional sample, but not in consecutive samples
Water in the Distribution System		
Fecal Coliforms	0	
Coliforms	0	in 95% of samples examined throughout the year – in the case of large supplies when sufficient samples are examined
Coliforms	3	in an occasional sample, but not in consecutive samples
Unpiped Water Supplies (Untreated Sources)		
Fecal Coliforms	0	
Coliforms	10	should not occur repeatedly; if occurrence is frequent and if sanitary protection cannot be improved, an alternative source must be found, if possible

consists of substances other than the coagulating agents. Even under these conditions, a few plant seeds have been found to act as effective coagulants. Among others, seeds from species of the family *Moringaceae*, e.g., *Moringa oleifera* and *Moringa stenopetala*, and also the *Loganiaceae*, e.g., *Strychnos potatorum*, have been found suitable. In fact, even before scientific confirmation of the coagulating properties of these seeds, the village women of Sudan had been using suspension of *M. oleifera* seed for many centuries. Reference to the use of *S. potatorum* seed for clarifying muddy water is available in Sushruta Samhita of the first century AD and it is still in use in the villages of Maharashtra and Tamil Nadu in India.

Among different species of *Moringaceae*, *M. oleifera*, commonly known as " drumstick tree " or " horseradish tree ", is the most widely occurring tree. Although it is native to northern India, it has become pantropical because of its many uses. It seems likely that rare *Moringaceae* species also contain natural coagulants (Jahn, 1986) that may yet be of practical interest, but most studies have been conducted with *M. oleifera* seed and, to some extent, with *M. stenopetala* seed which are relatively abundant. Used at optimum dosages (40–200 mg/L), according to the raw water types, *M. oleifera* seed removed visible turbidities from the Blue Nile water of 30–10,000 NTU in 1 or 2 hours with dosages similar to those of alum; however, it was found to be relatively less effective in clarifying water with turbidities < 20 NTU (Jahn, 1986).

The number of isolated water-soluble basic polypeptides (molecular weight 6-16 kDa) amounted to six in *M. oleifera* and nine in *M. stenopetala*. As *M. stenopetala* consistently outperformed *M. oleifera* on an equivalent weight basis for the White Nile water, it is most plausible that the chemical differences between the two *Moringa* coagulants in terms of the polypeptides were responsible for the difference in their clarification efficiency (Jahn, 1988). The study also indicated the robustness of treatment with *M. oleifera* seed as nearly maximum clarification was maintained over a relatively wide range of the seed dosage. This criterion is considered helpful in domestic water purification.

For microorganism removal, *M. oleifera* seed performed satisfactorily in the sense that raw waters treated with optimum dosages of the seed showed 98-100% reduction of fecal coliforms in 1 or 2 hours after coagulation when the residual turbidities had dropped to < 10 NTU (Jahn, 1988). The relative reduction of bacteria was less for lower degrees of raw water pollution even though the absolute counts after treatment were low. For further improvement of the microbiological quality following coagulation with *M. oleifera* seed, after-treatment by sand filtration has been suggested and a domestic coagulation-filtration unit with a meshed sand filter has been designed by the University of Khartoum, Sudan (Jahn, 1981).

Moringa seeds typically contain 4(α-L-rhamnosyloxy) benzyle isothiocyanate, a drug with bactericidal and fungicidal properties

(Eilert *et al*, 1981). However, the concentration of *Moringa* seed and hence the concentration of isothiocyanate normally used in domestic water purification is too small to kill bacteria (Jahn, 1981). Presumably, physico-chemical mechanisms effective in removal of the clay turbidity were responsible for the observed bacterial removal by *M. oleifera* seed.

Virus removal by *M. oleifera* seed was studied in South Africa by monitoring removal of naturally occurring coliphages (500 PFU/mL) from the Apis river water. Optimum coliphage removal was in the range 40–50% and paralleled the removal of naturally occurring fecal coliforms (Grabow *et al*, 1985).

Detailed studies on the clarifying action of *S. potatorum* seed were undertaken by the National Environmental Engineering Research Institute, Nagpur (Sen and Bulusu, 1962; and Bulusu and Sharma, 1965). It was observed that, if used as primary coagulant, low-turbidity waters required a more critical adjustment of the optimum dose and overdosing resulted in increased residual turbidity. Infrared spectra of the seed powder showed presence of carboxyl surface groups indicating its possible coagulating action as an anionic polyelectrolyte and a zeta potential study further suggested this (Chaudhuri *et al*, 1989). In jar test of three Lower Ganga canal water samples (pH 8.4; turbidity and heteretrophic plate count 500 NTU and 1400 CFU/mL, 300 NTU and 173 CFU/mL, and 150 NTU and 390 CFU/mL) with *S. potatorum* seed as prime coagulant, supernatant turbidity of 30, 25 and 20 NTU and bacterial removal of 42, 53 and 41% were

achieved at *S. potatorum* seed dosages of 2, 2 and 1 mg/L, respectively. However, considerable increase in supernatant plate count was observed in 24 hours when the clarified water was stored in contact with the sludge (Chaudhuri *et al*, 1989).

Another novel approach of applying natural coagulants to clarify water is their use as co-coagulants or coagulant aids with alum. Co-coagulation, using *M. oleifera* seed and alum simultaneously, showed significant improvement in coagulation ability and floc characteristics even at low turbidities and resulted in reduction in alum dose in the range 50-80% (Sutherland *et al*, 1989). In jar test of Lower Ganga canal water (pH 8.4; turbidity 500 NTU; heterotrophic plate count 2100 CFU/mL) with *S. potatorum* seed and alum as co-coagulants, supernatant turbidity and plate count were 5 NTU and 190 CFU/mL at 0.5 mg/L of *S. potatorum* seed with 20 mg/L alum, and 2 NTU and 50 CFU/mL at 0.5 mg/L of *S. potatorum* seed with 40 mg/L of alum, respectively (Chaudhuri *et al*, 1989). As per the observations of Sen and Bulusu (1962), residual turbidity will be still lower if *S. potatorum* seed is used as a coagulant aid and added 2-3 minutes before the addition of alum. For *Moringa* seed also, if the seed suspension is added 30-60 seconds prior to the addition of alum, the turbidity removal is better than when the seed suspension or alum is used alone (Jahn, 1986).

Because of the overall potential of the *Moringa* seeds for domestic water purification in rural settings, several developing countries are introducing the technology at the village level. In

1986, Yayasan Dian Desa of Indonesia collaborated with Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) of Germany to start a pilot project for dissemination of the water purification technology using *M. oleifera* seed in rural communities of Indonesia (Yayasan Dian Desa, 1989). To serve this purpose, Dian Desa has developed several audio-visual aids. In 1987, the Institute of Human Settlement, Bandung, Indonesia designed a low-cost water treatment scheme for individual household —— mixing *M. oleifera* seed powder (1 seed/L for turbid water and 6 seeds/L for coloured water) with raw water by 5-10 minutes of hand-stirring followed by 1-2 hours of settling and filtration through sand (35 cm; 0.3-1.2 mm) and charcoal (35 cm; 0.5-1.0 cm) (Setyawaty, 1989). *M. oleifera* seed has been used recently, either alone or in combination with alum, in existing low-volume water treatment works in Malawi (Sutherland, 1989) and in a pilot plant in the rural areas of Burundi (Käser, 1989). Based on the results of a laboratory study by Abu-Ghararah (1983), using *S. potatorum* seed to substitute synthetic polymer in direct filtration (filtration following coagulant dosing of a low-turbidity water without subsequent sedimentation), it appears that such a method of coagulation-filtration is useful in small water treatment plants or domestic water purification in rural areas of developing countries where *S. potatorum* seed is abundantly available. The Voluntary Health Association of India (VHAI) has initiated efforts to popularise the use of *S. potatorum* and *M. oleifera* seeds in domestic water purification in rural areas.

3. SCOPE OF THE PRESENT STUDY

Both quality and quantity of water supply are significant for the protection of public health. The need for quality improvement of surface water supplies on which a vast majority of the rural population in developing countries still depends is well recognised. Needless to say, this is a challenging task and a plausible solution would be reintroduction of traditional domestic water purification methods such as the use of natural coagulants. In India, two well-known natural coagulants (seeds of *S. potatorum* and *M. oleifera*) are available. The seed of *S. potatorum* is in use in rural areas of Tamil Nadu and Maharashtra in domestic water purification; however, the seed of *M. oleifera*, commonly used as a natural coagulant in African villages, is not popular in India even though it is abundant all over the country. The present study was directed towards a comparative evaluation of the performance of the seeds of *S. potatorum* and *M. oleifera* in domestic water purification. The study was undertaken along the following lines.

a. Performance evaluation of *S. potatorum* and *M. oleifera* seeds in batch coagulation-sedimentation test in terms of turbidity, bacteria (heterotrophic plate count) and virus (poliovirus) removal. Alum was used as a reference.

b. Sand filtration of coagulant (*S. potatorum* or *M. oleifera* seed) dosed water (coagulation-filtration test) to asses further improvement in turbidity, bacteria (heterotrophic

plate count and fecal coliforms) and virus (poliovirus) removal.

c. Infrared spectra and zeta potential study to probe into the nature of the coagulating action of the seeds.

4. MATERIALS AND METHODS

4.1 Materials

4.1.1 Water

Water from the Lower Ganga canal, Kanpur was used as raw water for the study. The water had the following characteristics: turbidity 15-120 NTU; pH 8.05-9.12; alkalinity 76-110 mg $CaCO_3$/L; and heterotrophic plate count (plate count agar) 212-380 CFU/mL. Canal bottom sediment was collected and used to augment the turbidity of the raw water to the required level when needed. Settled domestic wastewater was added (1 mL/L) to the raw water to introduce fecal coliforms (220-500 MPN/100 mL) when required; this produced a heterotrophic plate count (R2A agar) of 245-493 CFU/mL. Raw water for the tests to asses virus removal was the canal water seeded with poliovirus type 1 (Sabin), obtained from the ICMR Enterovirus Research Centre, Bombay.

4.1.2 Coagulants

Dried *S. potatorum* seeds, obtained through the Maharashtra Engineering Research Institute, Nasik, were powdered by pounding and the powder was sieved through a 150 μm sieve. The sieved powder was weighed and transferred to a volumetric flask to prepare a 0.2% suspension with distilled water.

Dried *M. oleifera* seeds were collected in April-May, 1992 from the Indian Institute of Technology, Kanpur campus, seed wings and coats were removed and the kernels were inspected. Only the kernels that did not show signs of softening or extreme

dessication were weighed, thoroughly pounded and squeezed in a mortar. The somewhat pasty (the result of seed oils) powder was then transferred to a volumetric flask. A 2% suspension was made with distilled water and was vigorously shaken for 5 minutes as suggested by Jahn (1988).

The *S. potatorum* seed suspension had a brownish colour and was stored in a refrigerator up to 10 days as there is no mention in the literature about reduction in its efficiency with time. The *M. oleifera* suspension had a milky appearance and was prepared just before use. Any unused suspension was stored in a refrigerator up to 3 days and discarded thereafter as recommended by Jahn (1988).

Alum used was reagent grade aluminium sulphate, $Al_2(SO_4)_3.16H_2O$, supplied by the Thomas Baker (Chemicals) Private Ltd., Bombay.

4.2 Methods

4.2.1 Bacteria and Virus Enumeration

Pour plate method, using plate count agar (35 $\pm$ 0.5^{o}C/48 $\pm$ 3 hours) or R2A agar (35 $\pm$ 0.5^{o}C/120 hours), was employed for heterotrophic plate count (HPC) as per the Standard Methods (1985). All plate counts were performed in duplicate and the mean values were reported as colony-forming units (CFU)/mL. The multiple tube technique, using lactose broth and EC broth, was employed for enumeration of fecal coliforms as per the Standard Methods (1985) and the values were reported as most probable number (MPN)/100 mL.

For virus enumeration, plaque assay (Smith and Gerba, 1982) on MA-104 cells, obtained from the National Facility for Animal Tissue and Cell Culture, Pune, was used. Each sample dilution was assayed in duplicate and the virus concentrations were reported as plaque-forming units (PFU)/mL.

4.2.2 Batch Coagulation-Sedimentation Test

Batch coagulation-sedimentation test (rapid mixing for 1 minute at 100 rpm, slow mixing for 20 minutes at 20 rpm and quiescent settling for 30 minutes) was conducted using a standard jar test apparatus (Phipps & Bird, Inc., Richmond, VA, U.S.A). Three raw water turbidities——low (ca. 20 NTU), moderate (ca. 40 NTU) and high (ca. 135 NTU)——were employed. Supernatant samples were analysed for turbidity and heterotrophic plate count (plate count agar). Supernatant samples were also filtered through Whatman No. 40 filter paper and the filtrate turbidity as well as time required for filtration of a 20 mL sample were recorded. According to Hudson and Wagner (1981), the filtrate turbidity gives an indication of the actual turbidity following coagulation-sedimentation-filtration and filtration time the filtrability of the coagulated-settled water.

Batch coagulation-sedimentation test to asses virus removal was conducted using a similar procedure. Poliovirus type 1 (Sabin) seeded (ca. 10^7 PFU/mL) canal water of low, moderate and high turbidities at observed optimum coagulant dosages for turbidity removal (based on supernatant turbidity) were employed. Raw water and supernatant samples were subjected to plaque assay

for virus enumeration.

4.2.3 Coagulation-Filtration Test

Locally available Yamuna sand, passing through a 710 μm sieve and retained on a 500 μm sieve (geometric mean size 0.6 mm), was washed thoroughly with clean water to remove the adhered impurities, dried and used as filter medium. A glass column, 100 cm long and 5.08 cm internal diameter, was used as the test filter with a filter medium depth of 50 cm. The lower end of the filter column was reduced to a diameter of 0.8 cm and a manual flow control arrangement (glass stopcock or rubber tubing with a screw-clamp) was attached.

A set procedure for a filter run consisted of dosing 10 litres of raw water of low turbidity (ca. 20 NTU) with *S. potatorum* or *M. oleifera* seed, hand-stirring for 2-3 minutes and allowing the coagulant-dosed water to pass through the test filter from an overhead (2 m above the filter outlet) bottle at an initially adjusted rate of 9.8 $m^3/m^2/h$. Daily two filter runs (morning and evening) were conducted and the filter medium remained submerged in the intervening period. Observed optimum coagulant dosages for turbidity removal (based on supernatant turbidity in batch coagulation-sedimentation test) were employed as recommended by Adin and Rebhun (1974). Both raw water and effluent samples of the morning runs were analysed for turbidity, heterotrophic plate count (R2A agar) and fecal coliforms, whereas the samples of the evening runs were analysed for turbidity only. Backwashing of the test filter was accomplished at 50% bed

expansion using tap water.

Using a similar procedure, following backwashing and at least two normal filter runs, coagulation-filtration test to asses virus removal was conducted by challenging the filter with coagulant-dosed canal water of low turbidity (ca. 20 NTU) seeded (ca. 10^6 PFU/mL) with poliovirus type 1 (Sabin). Influent and effluent samples were subjected to plaque assay for virus enumeration.

4.2.4 Infrared (IR) Spectra

Equal amount of *S. potatorum* or *M. oleifera* seed powder was mixed with a small amount of potassium bromide, pounded in a mortar and pellets were prepared. Using these pellets, IR spectra were obtained between wavenumbers 4000 and 670 cm^{-1} in a Perkin-Elmer 1320 infrared spectrophotometer.

4.2.5 Zeta Potential Measurement

Low turbidity (ca. 20 NTU) Lower Ganga canal water samples were dosed with *S. potatorum* or *M. oleifera* seed and following rapid mixing for 1 minute at 100 rpm, average mobility of the particulates was measured under varied field strengths using an electrophoresis apparatus (Rank Brothers, Bottisham, Cambridge, England). Average zeta potential was computed using the Smoluchowski formula

$$\zeta = V\eta/E\varepsilon$$

where, ζ = zeta potential; V = particle velocity; η = kinematic viscosity of water; E = applied field strength; and ε = dielectric constant of water.

5. RESULTS AND DISCUSSION

Results are presented in graphical or tabular form and a discussion of the results follows each phase of the experimental work.

5.1 Batch Coagulation-Sedimentation Test

The first phase of the study was devoted to performance evaluation of *S. potatorum* and *M. oleifera* seeds in batch coagulation-sedimentation test. Alum was used as a reference. Experiments were conducted at low (18-21 NTU), moderate (38-42 NTU) and high (130-135 NTU) raw water turbidities. Supernatant samples were analysed for turbidity and heterotrophic plate count as well as for the time required for filtration of a 20 mL sample through Whatman No. 40 filter paper and filtrate turbidity. Performance of *S. potatorum* and *M. oleifera* seeds and alum is presented in Fig. 1 (low raw water turbidity), Fig. 2 (moderate raw water turbidity) and Fig. 3 (high raw water turbidity). Compared with alum (30-50 mg/L), the optimum dose range (based on supernatant turbidity and plate count bacteria removal) was much lower for *S. potatorum* seed (1.0-2.0 mg/L), and higher for *M. oleifera* seed (150-250 mg/L).

According to Chaudhuri *et al* (1989), the optimum dose and supernatant turbidity, using *S. potatorum* seed with Lower Ganga canal water of turbidity 150 NTU, were 1 mg/L and 20 NTU, respectively. In the present study, batch coagulation-sedimentation test, using *S. potatorum* seed at high (130-135 NTU) raw water turbidity, showed optimum dose (based on supernatant

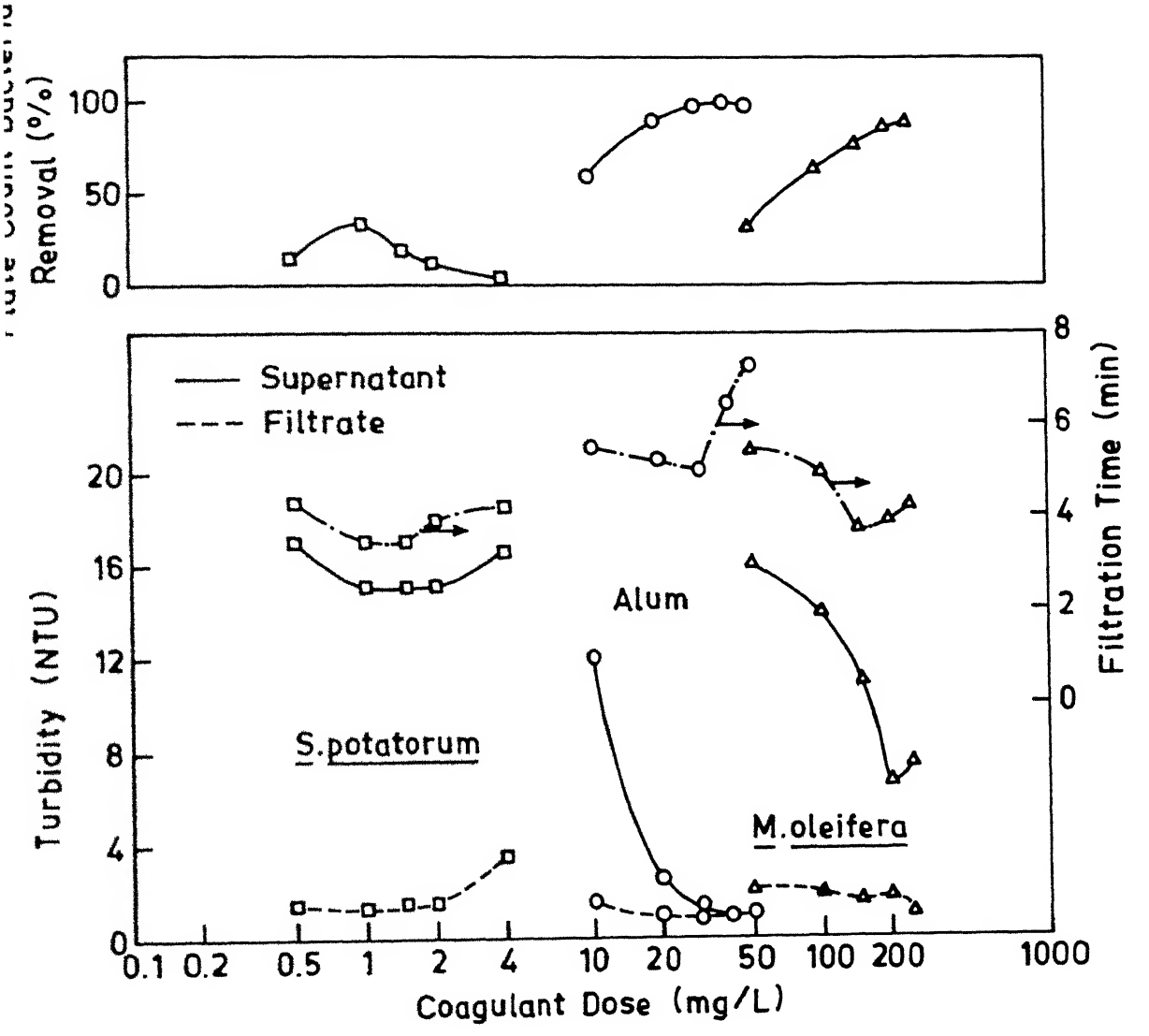

Fig. 1. Performance of S.potatorum and M.oleifera Seed and Alum in Batch Coagulation-Sedimentation Tes at Low Raw Water Turbidity.

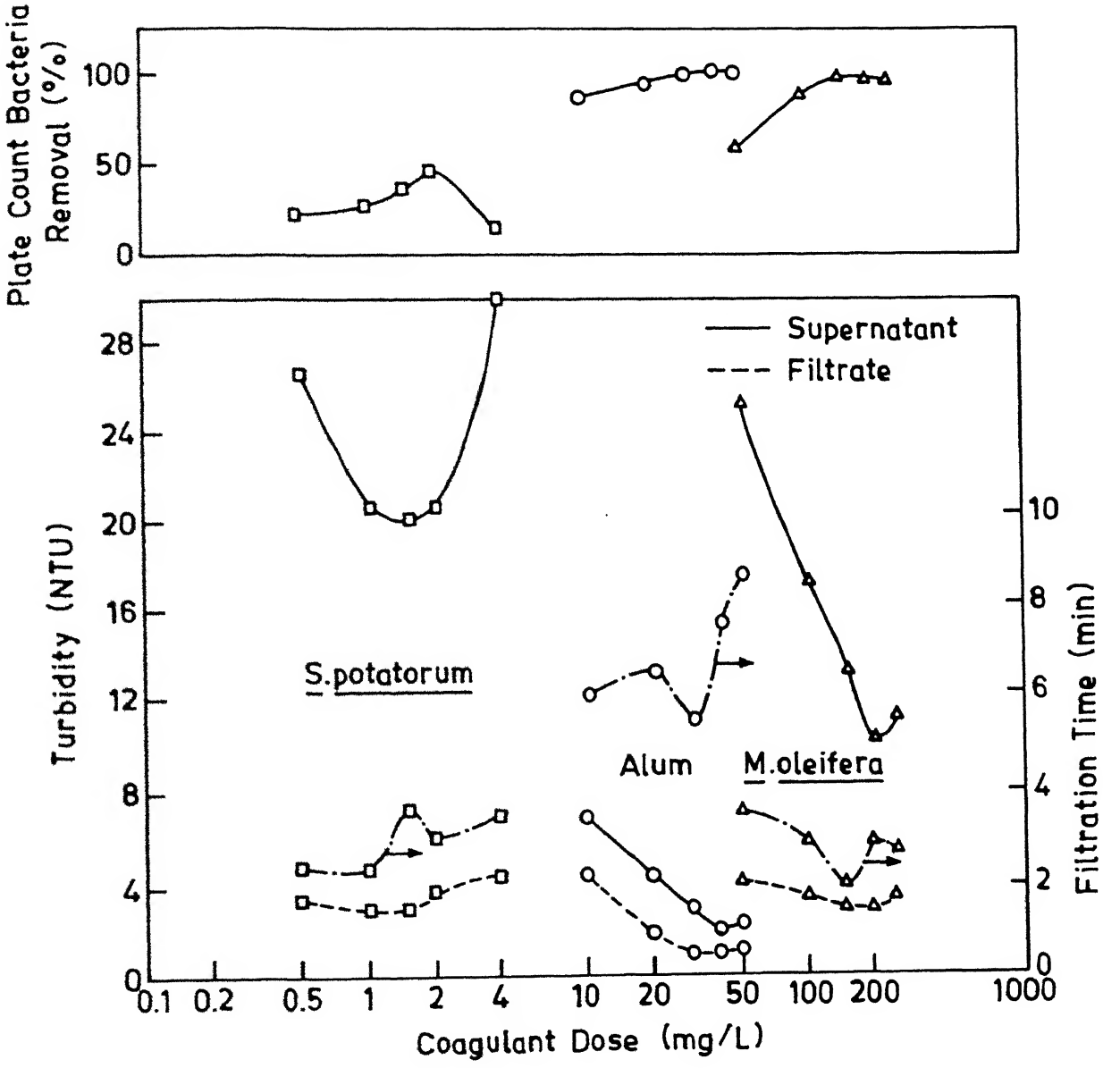

Fig. 2. Performance of S.potatorum and M.oleifera Seeds and Alum in Batch Coagulation-Sedimentation Test at Moderate Raw Water Turbidity.

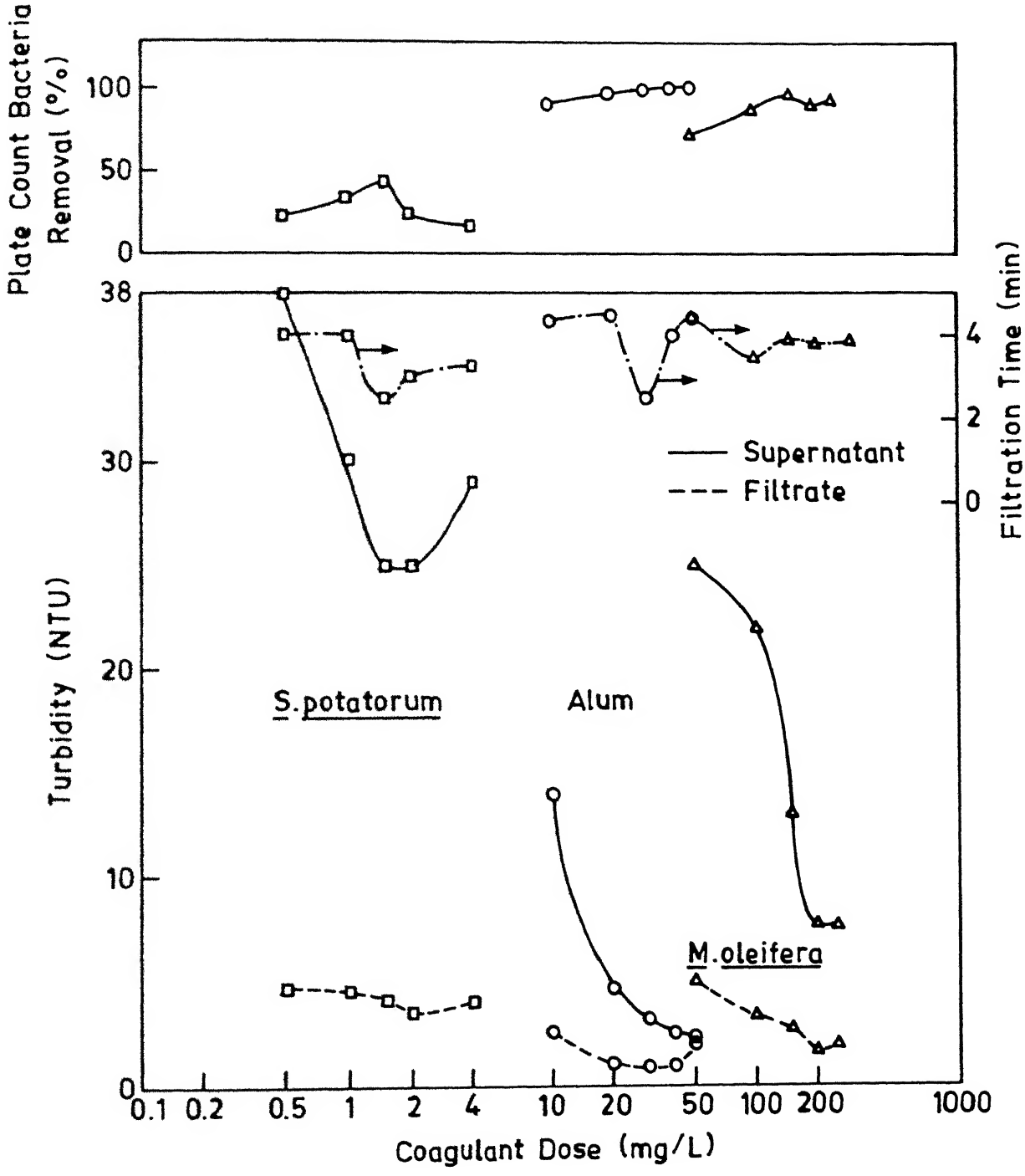

Fig. 3. Performance of S.potatorum and M.oleifera Seeds and Alum in Batch Coagulation-Sedimentation Test at High Raw Water Turbidity.

turbidity) and supernatant turbidity of 1.5 mg/L and 25 NTU, respectively (Fig. 3). Plate count bacteria removal was 43.75% and comparable with 41% removal observed by them.

Optimum dose (based on supernatant turbidity) of *M. oleifera* seed was 200 mg/L for all raw water turbidities. This was in line with the findings of Jahn (1986) that the optimum dose of *M. oleifera* seed was 40-200 mg/L according to the raw water types. Tests, conducted by Jahn (1988), using *M. oleifera* seed at optimum dose, showed 98-100% reduction in fecal coliforms which was reflected in the present study showing 84.38-94.41% removal of plate count bacteria (Fig. 1-3).

Considering overall performance, turbidity removal (supernatant) by *M. oleifera* seed was comparatively lower than that showed by alum, but it was higher than that by *S. potatorum* seed. Filtrate turbidity and filtration time were almost same for both seeds. Plate count bacteria removal by *M. oleifera* seed and alum was comparable, whereas it was lower for *S. potatorum* seed and almost paralleled turbidity removal for all three coagulants.

Performance of *S. potatorum* and *M. oleifera* seeds and alum in batch coagulation-sedimentation test at optimum dose (based on supernatant turbidity) are presented in Table 2. It is seen that when the seeds were used in coagulation-sedimentation test without subsequent filtration, the residual turbidity in all cases was > 5 NTU (guideline value recommended by WHO). From the filtrate turbidity values, it became apparent that addition of a filtration step would improve the performance of the seeds considerably.

Table 2. Performance of *S. potatorum* and *M. oleifera* Seeds and Alum in Batch Coagulation-Sedimentation Test at Optimum Dose

Raw Water Turbidity	Parameter	*S. potatorum*	*M. oleifera*	Alum
Low (18-21 NTU)	Optimum Dose (mg/L)	1.50	200.00	40.00
	Supernatant Turbidity (NTU)	15.00	6.50	0.95
	Filtrate Turbidity (NTU)	1.50	1.70	0.95
	Plate Count Bacteria Removal (%)	19.50	84.38	97.96
Moderate (38-42 NTU)	Optimum Dose (mg/L)	1.50	200.00	40.00
	Supernatant Turbidity (NTU)	20.00	10.00	2.00
	Filtrate Turbidity (NTU)	3.00	3.00	1.00
	Plate Count Bacteria Removal (%)	35.53	94.41	98.94
High (130-135 NTU)	Optimum Dose (mg/L)	1.50	200.00	40.00
	Supernatant Turbidity (NTU)	25.00	7.70	2.50
	Filtrate Turbidity (NTU)	4.00	1.70	0.90
	Plate Count Bacteria Removal (%)	43.75	89.39	99.33

Data on poliovirus removal by *S. potatorum* and *M.oleifera* seeds in batch coagulation-sedimentation test at optimum dose (based on supernatant turbidity) are presented in Table 3. Both seeds performed well effecting more than three log virus removal irrespective of raw water turbidity. A study conducted by Grabow *et al* (1985) reported 40-50% removal of indigenous coliphages by *M. oleifera* seed. No information on virus removal by *S. potatorum* seed is available. More tests are needed to evaluate the performance of the seeds in removing enteric viruses (both model and indigenous) from water.

5.2 Coagulation-Filtration Test

Based on the results of a laboratory study by Abu-Ghararah (1983), using *S. potatorum* seed to substitute synthetic polymer in direct filtration (filtration following coagulant dosing of a low-turbidity water without subsequent sedimentation), it appeared that such a method of coagulation-filtration would be useful in small water treatment plants or domestic water purification in rural areas of developing countries. A domestic coagulation-filtration unit with a meshed sand filter has been designed by the University of Khartoum, Sudan (Jahn, 1981) for improved microbiological quality of *M. oleifera* seed treated water. Another low-cost water treatment scheme has been designed by the Institute of Human Settlement, Bandung, Indonesia using coagulation with *M. oleifera* seed by hand-stirring followed by dual media filtration (Setyawaty, 1989). Based on this background and since *S. potatorum* and *M. oleifera* seeds did not improve the

Table 3. Poliovirus Removal in Batch Coagulation-Sedimentation Test

Coagulant	Dose (mg/L)	Raw Water Turbidity (NTU)	Removal (%)
S. potatorum	1.5	20	99.92
		35	99.95
		140	99.97
M. oleifera	200.0	20	99.93
		35	99.94
		140	99.96

Raw water poliovirus concentration 5.80×10^{7} PFU/mL

water quality to the required level in batch coagulation-sedimentation test, a filtration step was added. The coagulation-filtration test consisted of hand-stirring of coagulant-dosed water for 2-3 minutes and filtering the water through a sand filter without settling. It was thought that this would be a feasible method for domestic water purification.

Daily two filter runs were conducted and each filter run consisted of sand filtration of 10 litres of coagulant (*S. potatorum* or *M. oleifera* seed) dosed water. One raw water sample and effluent samples corresponding to 1, 5 and 9 litres were collected and analysed for turbidity, heterotrophic plate count and fecal coliforms for the morning runs and only for turbidity in the evening runs.

5.2.1 Performance of *S. potatorum* Seed

In all, thirty-six filter runs (360 litres throughput) were conducted at the optimum dose of 1.5 mg/L (based on supernatant turbidity in batch coagulation-sedimentation test) and the raw water and effluent characteristics are presented in Fig. 4. Consistent effluent characteristics were observed following the first two runs and the best effluent characteristics (turbidity 0.37-1.60 NTU; plate count bacteria 4-13 CFU/mL; fecal coliforms 3-11 MPN/100 mL) were observed from the third to twenty-fourth runs (20 litres to 240 litres). Effluent quality in terms of all parameters tested worsened thereafter. Backwashing (50% bed expansion) was carried out after the thirtieth run (300 litres throughput). Following backwashing, six more runs (60

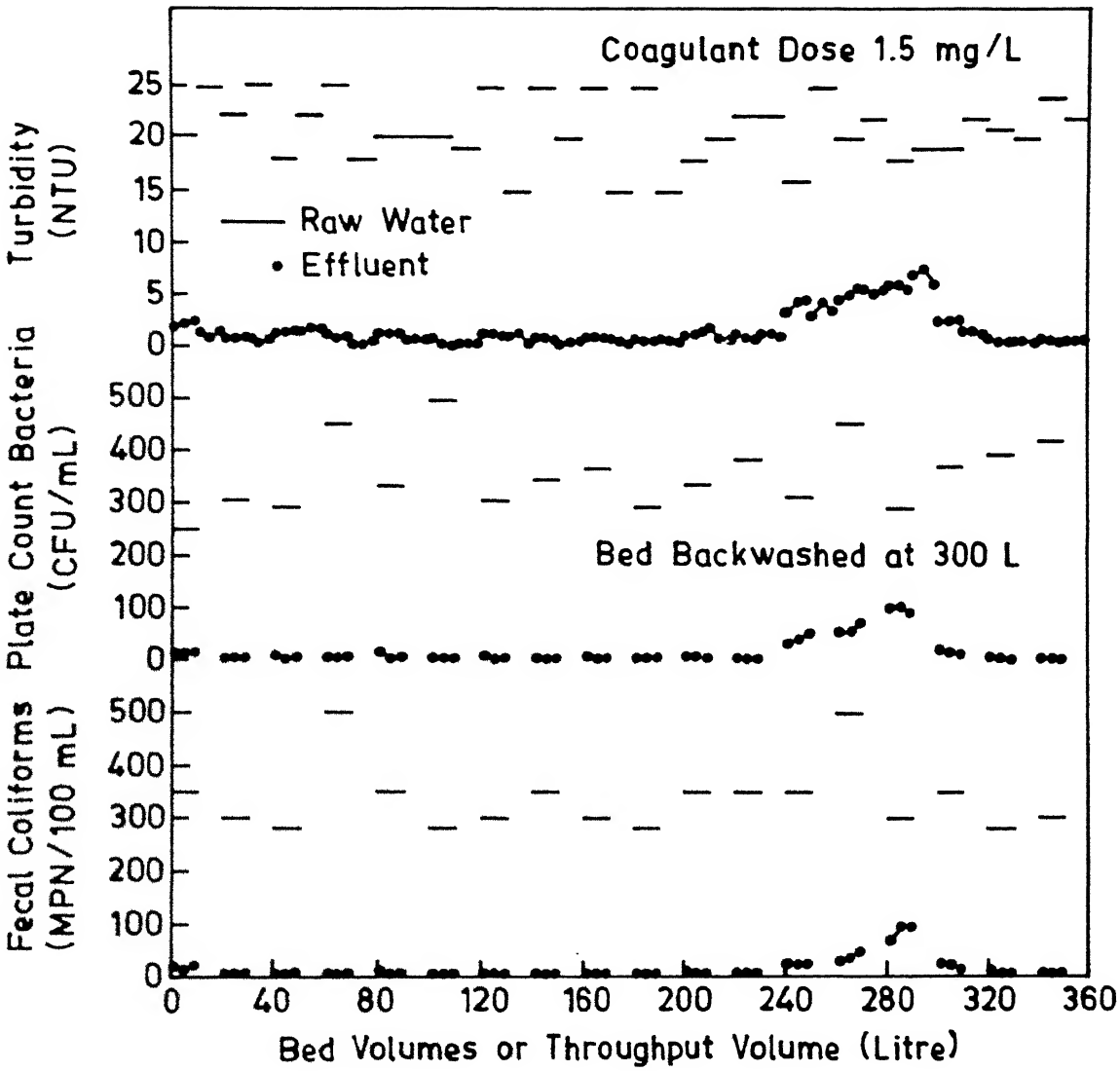

Fig. 4. Performance of S.potatorum Seed in Coagulation-Filtration Test.

litres throughput) were conducted and the performance was as before. Coagulation-filtration tests, using *S. potatorum* seed dosages of 1.0 and 2.0 mg/L, were conducted to asses the effect of slight variation in dose from the optimum. Eight filter runs (80 litres throughput) were conducted in each test and the results are presented in Fig. 5. The lower dose performed similar to the optimum dose, whereas the dose of 2 mg/L showed poor effluent characteristics for a much longer initial duration.

Following backwashing and atleast two normal filter runs, a coagulation-filtration test was conducted for evaluation of the performance of *S. potatorum* seed in virus removal and the results are presented in Table 4. In a five-litre filter run, effluent samples (1.5 and 3.5 litres) showed greater than two log removal of the seeded poliovirus.

5.2.2 Performance of *M. oleifera* Seed

Filter runs conducted with *M. oleifera* seed suspension resulted in clogging of the test filter within the first six filter runs (60 litres throughput), presumably because of the higher dose (200 mg/L). A batch coagulation-sedimentation test, conducted using the settled solids of *M. oleifera* seed suspension, yielded very meager removal of turbidity. Since only the soluble fraction of the suspension was effective, filter runs were conducted using a 30-minute settled *M. oleifera* seed suspension.

In all, twenty-two filter runs (220 litres throughput) were conducted at the optimum dose of 200 mg/L (based on supernatant turbidity in batch coagulation-sedimentation test) and the raw

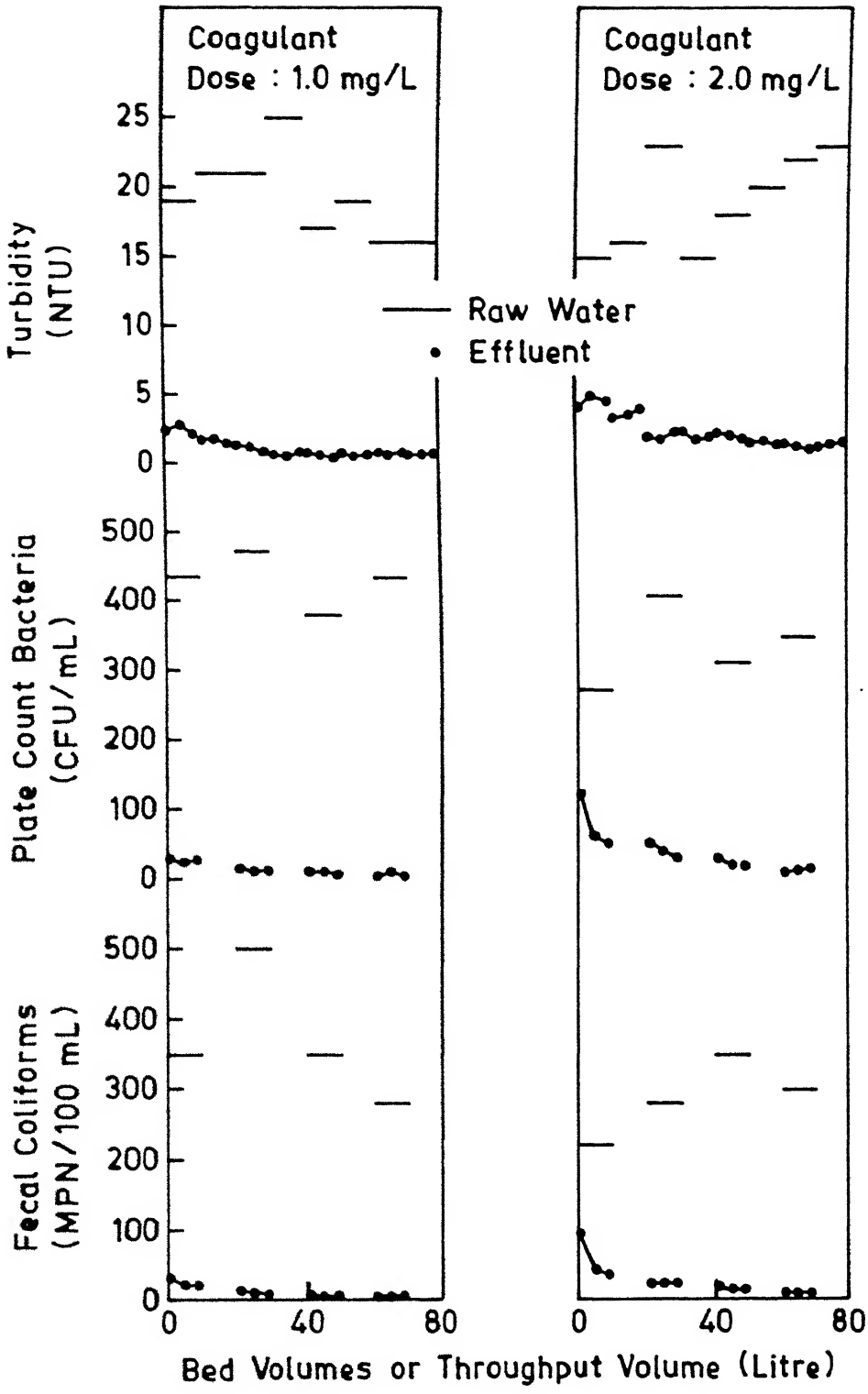

Fig. 5. Performance of S.potatorum Seed in Coagulation-Filtration Test at Dosages Lower and Higher than Optimum.

Table 4. Poliovirus Removal in Coagulation-Filtration Test

Coagulant	Dose (mg/L)	Raw Water		Removal (%)	
		Turbidity (NTU)	Virus (PFU/mL)	1.5 L	3.5 L
S. potatorum	1.5	22	2.37×10^6	99.36	99.56
M. oleifera	200.0	24	5.00×10^6	99.92	99.99

water and effluent characteristics are presented in Fig. 6. Consistent effluent characteristics were observed following the first two runs and the best effluent characteristics (turbidity 0.3-1.1 NTU; plate count bacteria 5-19 CFU/mL; fecal coliforms 4-20 MPN/100 mL) were observed from the third to tenth runs (20 litres to 100 litres). Effluent quality in terms of plate count bacteria and fecal coliforms worsened thereafter even though effluent turbidity remained low. Backwashing (50% bed expansion) was carried out after the sixteenth run (160 litres throughput). Following backwashing, six more runs (60 litres throughput) were conducted and the performance was as before.

Following backwashing and atleast two normal filter runs, a coagulation-filtration test was conducted for evaluation of the performance of *M. oleifera* seed in virus removal and the results are presented in Table 4. In a five-litre filter run, effluent samples (1.5 and 3.5 litres) showed three to four log removal of the seeded poliovirus.

5.2.3 Concluding Remarks

The coagulation-filtration test re-established the usefulness of *S. potatorum* and *M. oleifera* seeds in domestic water purification. For both seeds, the effluent turbidity level was below the recommended WHO guideline value of 5 NTU (WHO, 1984), and removal of the seeded poliovirus was greater than two log for *S. potatorum* and three to four log for *M. oleifera*. Effluent fecal coliform levels (3-11 and 4-20 MPN/100 mL) should not be of great concern because it is not usually possible to meet a zero

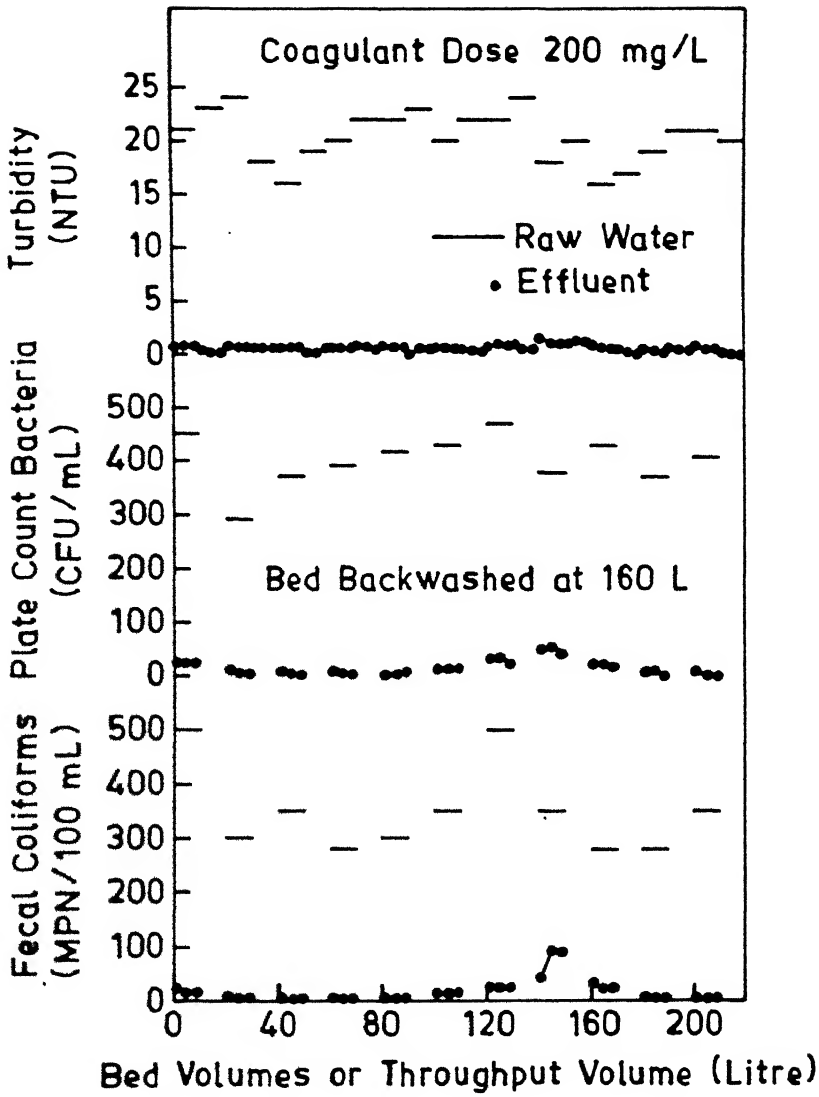

Fig. 6. Performance of M.oleifera Seed in Coagulation-Filtration Test.

fecal coliform standard without chlorination. It may be noted that the WHO guideline value of zero fecal coliforms for small untreated (unchlorinated) water supply (Table 1) had been questioned (Feachem, 1977) and an improved supply, providing with water up to 50 fecal coliforms/100 mL for instance, is considered to be a great advance in a developing country (Feachem, 1980).

5.3 Infrared (IR) Spectra and Zeta Potential Study

IR spectra (4000-600 cm^{-1}) of *S. potatorum* and *M. oleifera* seeds are presented in Fig. 7. The weak and broad vibrations at wavenumbers 1700 and 1620 cm^{-1} in the *S.potatorum* spectrum indicate presence of carboxylic acid groups which would be negatively charged at the pH range of natural water. According to Packham (1967), most of the natural polymers (polyelectrolytes) and their derivatives are based on a polysaccharide skeleton and have anionic properties derived from the presence of carboxyl groups. The spectrum of *M. oleifera* also shows presence of carboxylic acid groups. Further, vibrations at wavenumber ca. 1725 cm^{-1} indicate presence of ketonic/aldehyde groups which normally have a greater affinity to become positively charged.

A zeta potential study was conducted and the effect of *S. potatorum* and *M. oleifera* seed dose on the zeta potential of Lower Ganga canal water particulates (initial zeta potential -25.65 mV) is presented in Fig. 8. Increasing dosages (0.5-1.5 mg/L) of *S. potatorum* seed reduced the negative zeta potential to -17.18 mV at 1.5 mg/L and this corresponded to the optimum dose (based on supernatant turbidity) in the batch

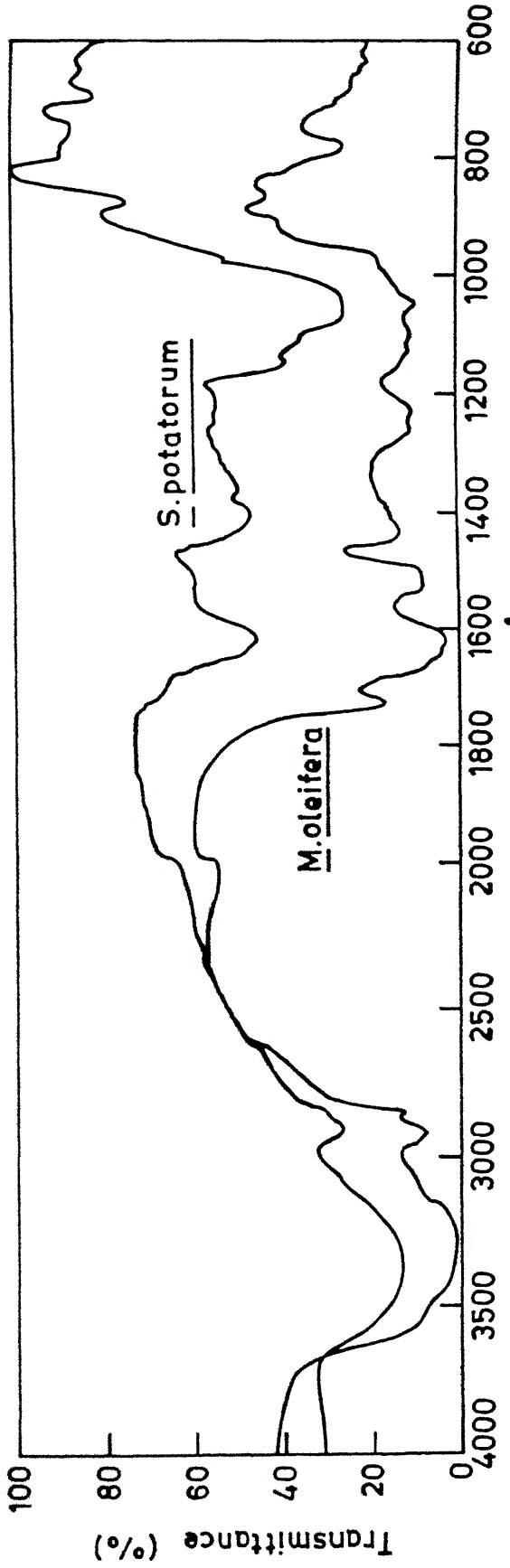

Fig. 7. Infrared Spectra of S.potatorum and M.oleifera Seed Powder.

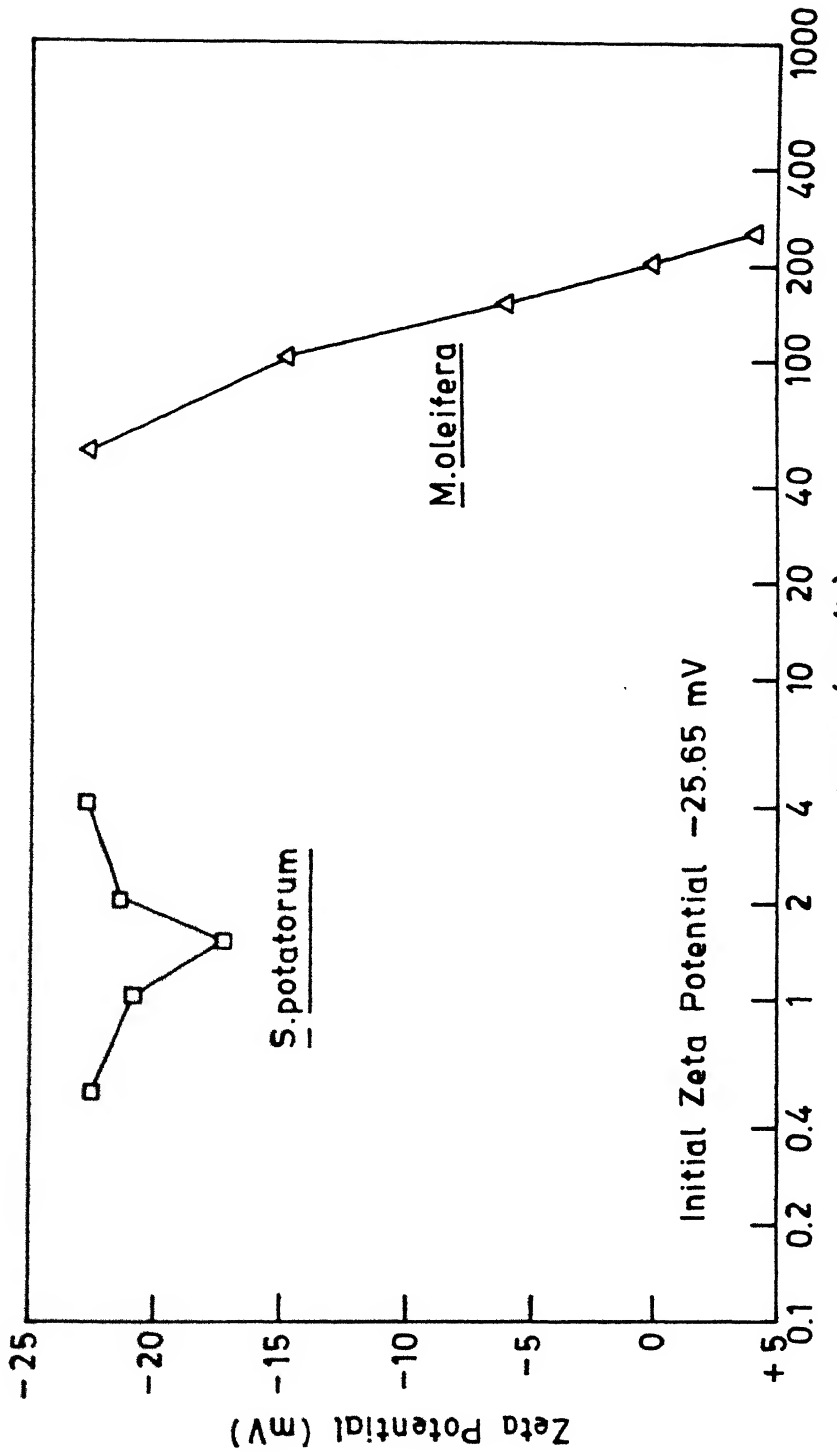

Fig. 8. Effect of S.potatorum and M.oleifera Seed Dose on Zeta Potential of Lower Ganga Canal Water Particulates.

coagulation-sedimentation test. At higher dosages (1.5-4.0 mg/L), the zeta potential became more negative. This pattern suggested an anionic nature of the *S. potatorum* seed polyelectrolyte and corroborated the indication of the IR spectrum. As observed by Packham (1967), it is difficult to explain coagulation of suspended particles carrying a net negative charge by anionic polyelectrolytes on the basis of the more classical theories of coagulation. La Mer and Healy (1963) developed a theory of interparticle bridging which has been applied to the coagulation of a number of mineral suspensions with polyelectrolytes—polymer molecules attach themselves to the surface of suspended particles at several adsorption sites with portions extending into the bulk of the solution and bridging takes place as a result of effective contacts between polymer segments and bond sites on other particles; if the polymer concentration is increased then the number of vacant adsorption sites is decreased until eventually a situation is reached where the vacant sites are so sparse that the suspension become stabilised. Black *et al* (1965) reported that the action of anionic polyelectrolytes is also influenced by coulombic forces and coagulation can take place if a sufficient concentration of calcium ions is present in the water.

Increasing dosages (50-200 mg/L) of *M. oleifera* seed steadily reduced the negative zeta potential to -0.125 mV at 200 mg/L and this corresponded to the optimum dose (based on supernatant turbidity) in the batch coagulation-sedimentation test. The zeta potential became positive (+4.06 mV) at 250 mg/L.

This pattern suggested a cationic nature of the *M. oleifera* seed polyelectrolyte. Even though the IR spectrum of *M. oleifera* seed shows presence of both negative and positive groups, the net charge can be negative or positive depending on their intensity. Black *et al* (1965) also showed that electrophoretic mobility of suspended particles was reduced considerably by small additions of cationic polyelectrolyte. Since the suspended particles in water tend to have a net negative charge, cationic polyelectrolytes would be expected to be most effective for removal of these particles (Packham, 1967). This has been clearly reflected in the present study showing higher removals (turbidity and heterotrophic plate count bacteria) by *M. oleifera* seed.

The IR spectra and zeta potential study delineated the nature of the "active coagulating principle" in *S. potatorum* and *M. oleifera* seeds. Observed wide difference in their optimum dosages was presumably due to a difference in the concentration of this principle in the seeds. Efforts were made to isolate the principles through isopropyl alcohol precipitation of the water-soluble component of the seed suspensions. For further instrumental analysis of the active principles, an attempt was made to redissolve the precipitates. The *S. potatorum* precipitate dissolved in water, whereas it was not possible to dissolve the *M. oleifera* precipitate. This portion of the study remained inconclusive and further investigation is warranted.

6. SUMMARY AND SUGGESTIONS FOR FUTURE WORK

A comparative evaluation of the performance of two natural coagulants (seeds of *Strychnos potatorum* and *Moringa oleifera*) was undertaken in terms of turbidity, bacteria (heterotrophic plate count and fecal coliforms) and virus (poliovirus) removal using the Lower Ganga canal water. In batch coagulation-sedimentation test, *M. oleifera* seed outperformed *S. potatorum* seed in the removal of turbidity and heterotrophic plate count bacteria, whereas both seeds showed excellent virus removal. Performance of both seeds, however, was commendable in coagulation-filtration test in removing turbidity, bacteria (heterotrophic plate count and fecal coliforms) and virus. Higher throughput was observed with *S. potatorum* seed before filter backwashing became necessary. This method (hand-stirring of the coagulant-dosed raw water for 2-3 minutes followed by filtration through a 50 cm sand column) appeared feasible for domestic water purification in rural areas of developing countries. Infrared spectra and zeta potential studies indicated the coagulating action of *S. potatorum* seed as an anionic polyelectrolyte and *M. oleifera* seed as a cationic polyelectrolyte.

The present work has re-established the usefulness of *S. potatorum* and *M. oleifera* seeds in domestic water purification. However, the following suggestions for future work are considered appropriate.

a. Performance evaluation of both coagulants under varied levels of water quality.

b. Removal of more significant model enteric viruses (rotavirus and hepatitis A virus) as well as naturally occurring enteric viruses in the coagulation-filtration test.

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